

Universität Hohenheim

Faculty of Agricultural Science

Institute of Animal Production in the Tropics and Subtropics

Animal Breeding and Husbandry

Prof. Dr. Anne Valle Zárate



Bachelor thesis

Assessment of water and milk quality in rural mixed crop-livestock farming systems: a case study of Lume and Siraro districts, Ethiopia

by

Marisa Spengler

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First supervisor: Prof. Dr. Anne Valle Zárate

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Abbreviations

CFU	Colony Forming Units
CPC	Coliform Plate Count
CSA	Central Statistical Agency of Ethiopia
EC	Electrical Conductivity
EU	European Union
IMViC	Indole reaction, Methyl red test, Voges-Proskauer test, Citrate utilization
Log ₁₀	Logarithm to base ten
N	Sample size
NTU	Nephelometric Turbidity Unit
SD	Standard deviation
SPC	Standard Plate Count
TAPC	Total Aerobic Plate Count
TDS	Total Dissolved Solids
TSI	Triple Sugar Iron
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

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1. Introduction

Water is an important resource, which supports all forms of life and plays multiple roles in the livelihood of people in many parts of the world. Specifically, in rural areas of developing countries like Ethiopia, water is used by people for both consumptive and productive activities. For the reason that every person depend on water supply, high quality water is an essential need. It is know that microbial contamination as well as the chemical composition of water can adversely affect human health and consequently guidelines for drinking water quality are given by the World Health Organization (WHO, 2011). However, water of such a good quality may not be available in rural areas of developing countries (Wolf et al., 2003) and farmers may be obliged to use poor quality water for human drinking as well as for different livestock operations, where it is recommended that exemplarily water for milk production should have standards equivalent to drinking water (Chye et al., 2003). People in rural parts of developing countries may not have access to advanced water sources and are forced to use water from rivers, ponds or the roadside for example, where water quality is not monitored at all.

Milk provides a quick and easy way of supplying nutrients for humans. However, due to this nutritious composition milk is also an excellent media for the growth of microorganisms, originating from contamination. It is a highly perishable product, which loses quality within a short period of time and - if not well kept - becomes unwholesome for human consumption and may be a carrier of pathogens. Raw milk or other milk products can implicate a number of food-borne diseases, whereby bacteria play an important role in transmission (Kivaria et al., 2006).

Lume and Siraro districts in Ethiopia represent two characteristic situations concerning water problems in developing countries. Relatively, surface water is available in Lume compared to Siraro, where high scarcity of water prevails. However, the available water in Lume is at danger of pollution due to industrial activities such as tanneries and abattoirs. The fact that water is either scarce or polluted may lead to the usage of water of impaired quality, which represents a risk to human health.

Furthermore, raw milk and traditional milk products are produced, processed and consumed in many households and the surplus, which is not needed by the family itself, is sold to neighbours and hotels or on a formal market to milk collection centres. Yet there is no quality assessment. As a consequence milk products of inferior quality and dangerous to health may

end up on a consumer level. In addition with the fact that raw milk and its products are commonly used without prior treatment milk consumption present a hazard to human health.

Therefore, the objective of this study was to assess the quality of water from sources and from households, which represent the places of water origin and consumption, respectively. It was concentrated on the physico-chemical and bacterial issues of water quality. Moreover, the prevalence of bacteria in quantity and quality of consumed milk products along the milk production chain was surveyed.

First of all chapter two is giving an overview of the relevant information about water and milk quality, which is known today and which is related to the assessed aspects of quality. The applied materials and methods of the survey are illustrated in chapter three. Subsequent chapter four is dedicated to the outcome of the study and finally the received information is imbedded and compared to connected literature in chapter five.

2. Literature review

2.1. Water quality

The crucial parameters determining water quality and the corresponding standards depend on the way water is used. Among the various guidelines there are specific directives for water used for human consumption, livestock, fisheries, irrigation and recreation (Scatena, 2000). According to the World Health Organization (WHO), the factors affecting human health include microbial, chemical and radiological aspects (WHO 2011). Impaired drinking water quality can have numerous causes, beginning with the groundwater level and reaching to the consumption. It was found, for instance, that chemical contaminants like arsenic in groundwater (Smith et al., 2000), storage conditions of drinking water in households (Wright et al., 2004), the shape of the vessels used for water storage (Quick et al., 1996) or the handling practices (Clasen and Bastable, 2003) influence water safety. Improvement can be achieved by applying treatment modalities such as heat, UV radiation, sedimentation, filtration or chemical methods on a household level (Clasen and Bastable, 2003) or by enhanced water supplies (WHO, 2000). However, Wright et al. (2004) emphasize, that recontamination is an issue often underestimated and that it is essential to ensure safety of water at the point of consumption.

2.1.1. Microbial hazards in water

Pathogens in water include bacteria, viruses, protozoa and helminths (WHO 2011, p. 231). Water functions as a passive carrier for microbes, which can cause water-borne diseases. Generally, they do not multiply in water (WHO 2011, p.232); they are spread via the faecal-oral route (Ashbolt, 2004) and induce infections in the gastrointestinal tract. But there are microorganisms, which increase in number in water, which are transferred by inhalation or skin contact or impair the respiratory tract and other parts (WHO 2011, p.232). Some bacteria of importance in developing countries, the corresponding diseases and origins are shown in Table 1 (Ashbolt, 2004).

Tab. 1 Relevant pathogens in water in developing countries

Name of Bacteria	Major diseases	Major reservoirs and primary sources
<i>Salmonella typhi</i>	Typhoid fever	Human faeces
<i>Salmonella paratyphi</i>	Paratyphoid fever	Human faeces
Other <i>Salmonella</i>	Salmonellosis	Human and animal faeces
<i>Shigella spp.</i>	Bacillary dysentery	Human faeces
<i>Vibrio cholera</i>	Cholera	Human faeces and freshwater zooplankton
<i>Yersinia enterocolitica</i>	Gastroenteritis	Human and animal faeces
<i>Campylobacter jejuni</i>	Gastroenteritis	Human and animal faeces
<i>Legionella pneumophila</i> and related bacteria	Acute respiratory illness (legionellosis)	Thermally enriched water
<i>Leptospira spp.</i>	Leptospirosis	Animal and human urine
<i>Various mycobacteria</i>	Pulmonary illness	Soil and water
Opportunistic bacteria	Variable	Natural waters

Source: Ashbolt, 2004

Among several indicators for water quality there are *Escherichia coli*, total coliforms, heterotrophic plate counts and *Clostridium perfringens* (WHO 2011, pp.294-301). The enumeration of *E.coli* as a faecal indicator is internationally well accepted and is the most commonly preferred method for quality surveillance, due to similarities in behaviour to such highly dangerous pathogens as *Vibrio cholera*, *Salmonella typhi* and *Salmonella paratyphi* (Ashbolt, 2004). According to the WHO guidelines for drinking water quality (2011, pp. 296-297) *E.coli* should not at all be detected in any 100 ml sample.

2.1.2. Chemical hazards in water

The WHO states five sources of chemical contaminants: (1) naturally occurring, (2) industrial sources and human dwellings, (3) agriculture, (4) water treatment or materials in contact with drinking water and (5) pesticides used in water for public health. The analyzed parameters in this study, their significance for health and main sources are summarized in Table 2.

Tab. 2 Relevant physico-chemical parameters of water quality

Parameter	Maximum	Health concern	Major source
pH	Not of health concern; usually recommended 6.5- 8.5	Indirect: disinfection, corrosion of pipes and appliance	
Temp.	No maximum Recommended < 20-25°C	Indirect: microbial growth, water treatment and acceptability	Natural; waste heat of devices
Turbidity	Not of health concern 5 NTU	Indirect: prevention UV disinfection, medium qualities for microbial growth	Soil run off, growth of algae
T.D.S	Not of health concern; For palatability < 1000 mg/l	May affect acceptability	Natural, industrial wastewater, salts for road de-icing
Calcium	Not of health concern For palatability < 100-300 mg/l	May affect acceptability	Natural sources
Magnesium	Not of health concern For palatability lower than Calcium	May affect acceptability	Natural sources
Copper	2 mg/l	Gastrointestinal tract	Corrosion of copper pipes
Manganese	0.4 mg/l (health) 0.1 mg/l (palatability)	Neurological effects (doubtful)	Natural sources (Earth crust)
Chromium	0.05 mg/l	toxic, carcinogenic	Earth crust
Fluoride	1.5 mg/l	Dental fluorosis, skeletal fluorosis	Earth crust
Nitrate	50 mg/l	Methaemoglobinaemia especially in bottle-fed infants	Natural, agriculture, wastewater, oxidation products of excreta
Sulphate	Not of health concern Recommended 500 mg/l For palatability 250 mg/l	extreme amounts laxative	Natural sources, industry

Source: adapted from Scatena, 2000 and WHO 2011, Ch.12

NTU: Nephelometric turbidity unit

Maximum values are indicated according to the guidelines for drinking water quality (WHO, 2011). For the detection of chemicals numerous methods are in place, including volumetric and colorimetric methods, gas and high-performance liquid chromatography, flame atomic absorption spectroscopy and others (WHO 2011, Ch. 12). In general threshold values are given for contaminants, which are occurring in drinking water in health affecting amounts or rarely for those influencing acceptability such as taste, odour and colour.

2.2. Milk quality

2.2.1. Origination of microbial contamination

Ordinary udder flora, inflammation of the mammary gland and the environment are the main sources of microbes in milk and milk products (Van Shaika et al., 2005). However, the amount of bacteria of raw milk which originates from a healthy udder is very low (usually <1000 colony forming units CFU/ ml) and is regarded not to be health impairing by itself (FAO, 2008; Yirsaw, 2004). The risk of contamination increases on the production chain while the milk moves towards the final consumer (Omore et al., 2004). In addition, milk is a highly nutritious medium for excellent microbial growth. Therefore only a small initial amount of bacteria is sufficient to lead to an inferior milk quality in a short time (Afif et al., 2008). Consequently, the achievement of safe end products is mainly dependant on the cow's health and the hygiene during milking and further storage and processing of the milk. Improper cleaning of the applied utensils, water of poor quality used for cleaning the udder or the milking and processing equipment and the milk handling personnel are examples for potential sources of contamination (Movassagh Ghazani et al., 2008). Moreover, on farm level feeds, faeces, bedding material and soil may transfer bacteria (Visser & Driehuis, 2009, p.2).

2.2.2. Microbial quality control

Microbes in milk can be assigned to two groups concerning their adverse effects: organism causing spoilage, due to enzymes, which hydrolyse milk components like proteins, and pathogens, which directly affect the health of consumers (Touch & Deeth 2009, p.67). At professionally milk-producing farms and dairy companies total microbial count, total coliform count, psychotrophs, which mainly are responsible for spoilage and pathogenic organisms are determined routinely. Among others indirect physical methods, metabolite testing, DNA-based inspections and especially microbial enumeration are common analysis practices (Belloque et al. 2009, p.94). Some of the bacterial counts are explained more detailed below.

Standard Plate Count (SPC)

Standard Plate Count, occasionally referred to as Total Aerobic Plate Count (TAPC), estimates the total number of aerobic bacteria in raw or processed liquid milk samples usually expressed in Colony Forming Units (CFU) per ml. In short, bacteria are grown on a non-selective media and incubated for 24-48 h at 37°C. All visible colonies are counted. For the test procedure in detail see chapter 3.3.2.1. High SPC results most often point to improper cleaning of milking equipment or warm storage conditions and casually prevalence of mastitis (Ruegg et al., 2008).

SPC is an internationally accepted reference method in monitoring milk quality and commonly used to grade raw milk (Table 3, Kurwijilla et al., 1992). In the United States of America, the Pasteurized Milk Ordinance, which is the responsible authority for hygiene of milk and milk products, requires a SPC of less than 100 000 CFU/ ml for milk of individual farms. However, depending on the supplied dairy company more stringent threshold values may exist (Ruegg et al., 2008). For selling of raw milk the maximum values are 20 000 CFU/ ml (UK, USA) and 100 000 CFU/ ml (EU) (Hickey 2009, pp. 112/ 116/ 124).

The advantages of the method are low costs, simplicity and high sensitivity, but the method fails to differentiate between pathogenic and non-pathogenic organisms. In summary, the test is a useful tool to get a primary overview of the bacterial load.

Tab. 3 Grades of raw milk depending on SPC

SPC in CFU/ ml	Grade
Not exceeding 200,000	Very good
200,000 – 1,000,000	Good
1,000,000-5,000,000	Fair
>5,000,000	Poor

Source: Kurwijilla et al. (1992)

Total Coliform Count or Coliform Plate Count CPC

In general, coliforms are mostly gram-negative, non-sporogenic, rod-shaped, aerobe and facultative anaerobic bacteria, which are capable of fermenting lactose. Genera belonging to this group are among others *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella* (WHO 2011, p. 295). The consequence of coliform prevalence in milk is primarily spoilage, due to enzymes, which are capable of hydrolysing milk components such as proteins. But some species may cause severe diseases (Huppertz & Kelly 2009, p.42).

The CPC functions in dairy processing as an indicator for the assessment of hygienic conditions during milk production and processing (Belloque et al. 2009, p.76). The sources of coliforms in milk are udders contaminated by bedding material, dung or soil or unclean milking equipments, for example (Visser & Driehuis, 2009, p.7). Determination of coliforms is commonly done by growth on selective media and subsequent counting (for details on the procedure see chapter 3.3.2.1). Maximum values for consumed raw milk are exemplarily 100 CFU/ ml and 10 CFU/ ml for Britain and the United States of America, respectively (Hickey 2009, pp.116/ 124).

Enumeration of Escherichia coli (E.coli)

Human and animal faeces carry *E.coli* in high amounts and contamination of milk, other food products and water occurs predominantly by faecal input (WHO 2011, p. 296). Consequently enumeration of *E.coli* is the method of choice when assessing sanitary conditions and additionally may be an indicator of prevalence of other enteric pathogens (Gran et al., 2001). Moreover, this species is occasionally isolated from milk of cows suffering from mastitis (Touch & Deeth 2009, p.51).

E.coli is not a relevant factor concerning aspects of spoilage, because sufficient growth leading to adverse effects is rarely achieved (Touch & Deeth 2009, p.49). Some *E.coli* strains, especially those producing shiga-toxins, are capable of causing several diseases with symptoms ranging from mild to severe. In disease outbreaks related to raw milk consumption *E.coli* was found to bring about gastroenteritis, hemorrhagic colitis, haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura, for example (Wells et al., 1991). Selective media is used for culturing *E.coli* and subsequent counting indicates the result, which is expressed commonly in CFU/ ml (for details on the procedure see chapter 3.3.2.1). There is no internationally valid threshold value for *E.coli* in raw milk or other milk products. However it is recommended, that pathogenic organisms should not be prevalent at all (Hickey 2009, Ch.5).

2.2.3. Specific bacteria associated with food-borne diseases

There is a wide range of possible pathogens in milk. However, most often *Listeria* spp., *Campylobacter* spp., *Salmonella* spp., *Escherichia* spp., especially *E. coli* O157 and *E. coli* O26, *Yersina* spp. and *Cryptosporidium* spp. are identified as pathogenic organisms (Touch & Deeth 2009, p.54). Table 4 illustrates important species, the major sources and the corresponding diseases.

Tab. 4 Relevant pathogens occurring in raw milk and milk products

Pathogenic species	Major source	Disease
<i>Staphylococcus aureus</i>	Mastitis	Food poisoning
<i>Streptococcus agalactiae</i> ,	Mastitis	Bacteraemia, meningitis
<i>Streptococcus dysgalactiae</i>	Mastitis	Meningitis
<i>Listeria monocytogenes</i>	Mastitis, environment	Listeriosis
<i>Campylobacter jejuni</i>	Environment (faeces)	Campylobacter enteritis
<i>Salmonella</i> spp.	Environment (faeces)	Salmonellosis
<i>Yersina enterocolitica</i>	Environment	Yersiniosis

The growth of disease-causing bacteria is inhibited naturally by competition with present non-pathogens or the application of treatment methods like cooling, pasteurisation, addition of CO₂ or bio-preservatives, for example (Touch & Deeth 2009, Ch.3).

3. Material and methods

3.1. Study area

Lume and Siraro, two districts of the Oromia Regional State in Ethiopia were determined for taking samples. Lume is located in the East Shewa Zone with its administrative centre, Modjo, located 70 km southeast of Addis Ababa, which is the capital city of Ethiopia. The area is estimated 675.15 km², of which approximately 60 km² are covered by water, and it comprises 35 villages. The total human population of the district is stated to be 133 438 by the Central Statistical Agency of Ethiopia (CSA), with 48.69% female and 51.31% male inhabitants and 67% live in rural areas (CSA, 2011). The district has a bi-modal rainfall distribution, receiving an annual average rainfall of 850 mm (Range: 500 mm to 1200 mm) with the main rainy season extending from June to September. Average temperature is ranging between 18°C and 28°C (Aschenafi, personal communication 22.08.2011).

Water resources used by people and livestock are rivers (both permanent and intermittent), lakes, wells (shallow and deep), springs and ponds. The CSA assumes that only 51-60% of the people have access to a protected and reliable water source (CSA, 2010).

Mixed crop-livestock production is the predominant farming system. Major crops grown in the area are *teff*, wheat, maize and barley with *teff* being the most common one. Livestock population in Lume is estimated to be 65 992 cattle, 11 832 sheep, 22 023 goats, 19 829 asses, 805 mules, 434 horses, 84 677 poultry and 2 412 beehives. Most of the 11 087 dairy cows are assigned to local breeds (98.6%), with the remaining cows belonging to crossbreeds with Holsteins or exotic breeds (Aschenafi, personal communication 22.08.2011). A common milk production chain is shown in Figure 1. In the milk producing households, 66.59% of the milk yield is consumed in the form of raw milk or processed milk products like *ergo*, *ayib*, yoghurt or skim milk. For *ergo* production, milk is kept at a warm place, which induces natural fermentation. The end product after approximately 24 hours is similar to sour milk. When *ergo* is further processed by churning, the fat is removed and finally the residue is heated up to 50°C. The cheese originating of this process is called *ayib* (Aschenafi, 2006).

Raw milk is commonly stored in smoked containers to achieve a better taste. In informal markets, 31.63% of the milk is sold to hotels and neighbours or is used as wages in kind, for example for servants. A minor proportion of milk (1.78%) is delivered to one of the three dairy associations in the district (CSA, 2008).

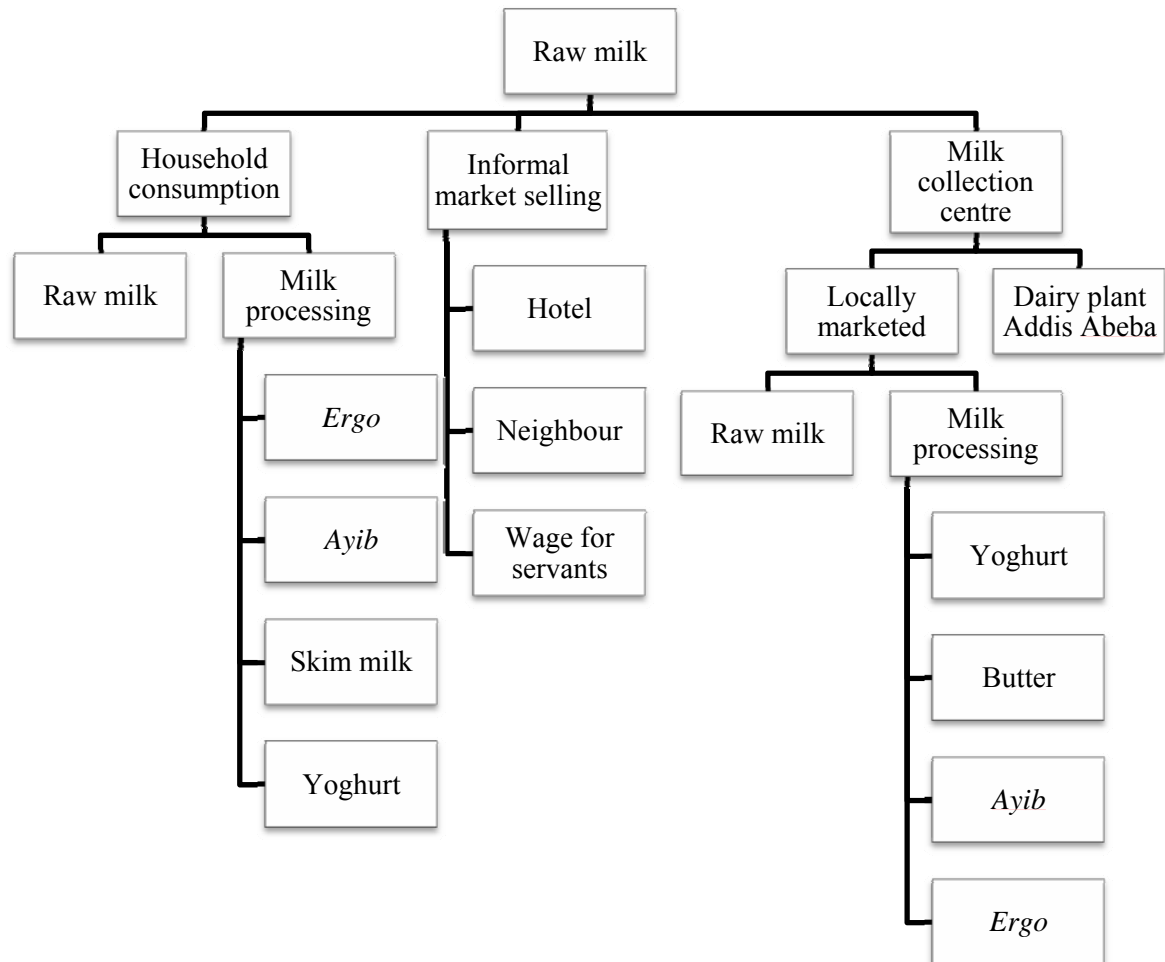


Fig. 1 The observed milk production chain in Lume, Ethiopia

Siraro district is located in the West Arsi Zone, Oromia Regional State, Ethiopia. Lokke is the administrative centre of the district and located approximately 353 km south of Addis Ababa. Siraro has an area of 599.35 km² and is divided into 28 village administrations with three town centres. The human population is assumed to be 167 932, splitting up into 31 003 households (CSA, 2011).

The district has a uni-modal rainfall pattern with an annual rainfall variation between 600 mm and 1500 mm and precipitation peaks in July, August and September. Accessibility for protected water sources is low (31-40%), and usually water is fetched from wells, cisterns, rivers, ponds or at the roadsides.

People in Siraro depend on a crop-livestock farming system for their livelihood. The main types of crops produced in the area include maize, haricot bean and potatoes. The livestock population of the district is counted to be 182 243 cattle, 11 339 sheep, 59 440 goats, 2 517 horses, 35 044 asses, 540 mules, 147 407 poultry and 14 759 beehives. Commonly, local breeds are kept and only a small number of the 51 797 dairy cows are hybrids. A large proportion of the produced milk (91.02%) is consumed by household members and only 1.77% is delivered to commercial dairy associations. The remainder is sold privately (CSA 2008).

3.2. Sampling procedure

The study was carried out as part of an ongoing PhD study. As a consequence, the sampling districts Lume and Siraro have already been chosen by Amenu et al. (2011). Water samples were taken from sources and from household containers, representing the places of water origin and water consumption, respectively.

In total 34 water sources were previously identified by the aforementioned study, comprising fourteen wells, seven borehole pumps, six rivers, three sewers of tanneries and abattoirs, two rainwater ponds, one cistern and one spring. In Lume, 24 sources were located whereas in Siraro ten water samples were taken. One litre of water was taken for physico-chemical analysis and 100 ml for quantitative *E.coli* and coliform analysis, each in a sterile sampling bottle. If borehole pumps ended in a tap, the sample was attained after at least ten seconds of water flow.

Households were randomly selected in two villages per district. In total, 44 households were visited in Lume and 77 households in Siraro. For *E.coli* and coliform counts, 100 ml of water was taken directly from the storage container of the family into a sterile sampling bottle.

PH, temperature, electrical conductivity (EC) and total dissolved solids (TDS) of all water samples were measured on the spot. The samples, destined for bacterial enumeration, were stored immediately after sampling in cooling bags at 4°C and analyzed within 24 hours. Samples, taken for physico-chemical analysis, were kept at room temperature between two and four weeks.

Milk samples were taken from different points of the local milk production chain. Therefore, the visited households were asked for milk directly from the udder, pooled raw milk and any other milk products, which they keep and consume. Milk samples were collected for bacteriological enumeration, isolation and presumptive identification of bacterial pathogens.

In total, 169 samples from quarters, 39 pooled raw milk samples from storage containers of the households and 19 samples from other milk products were obtained in. Other milk products comprised yoghurt, *ayib* and *ergo*. Additionally, six skim milk samples were attained in Siraro.

For quarter sampling, the teats were first cleaned from dry dirt and disinfected with ethanol (95% vol/vol). If the cow wasn't milked by the owner that day the first two stripes were discarded and about 10 ml of milk were gained in a sterile sampling bottle.

Pooled raw milk samples and other milk products were taken from the storage container of the household. Approximately 25 ml of milk were poured directly into a sterile sampling bottle.

In order to determine milk quality at the next level of the production chain, seven milk samples were directly obtained from transport container of farmers, which delivered to the milk collection centre in Lume by decanting around 25 ml of milk into sterile sampling bottle. After pooling the entire delivered milk of the sampling day, 25 ml were taken in a sterile sampling bottle from the storage container of the centre.

All milk samples were kept in a cooling bag for transportation, stored at 4°C in a refrigerator and analyzed within the next 72 hours.

Both milk and water samples were taken during the main wet season in July and August.

3.3. Sampling processing

3.3.1. Water

3.3.1.1. Bacterial enumeration in water

All water samples were analyzed for enumeration of *Escherichia coli* and total coliforms. Therefore heavily contaminated water was first diluted with Ringer's solution (Oxoid BR0052) up to 10^{-2} . Brilliance™ E.coli/ coliform selective agar (Oxoid CM 1046) was applied. The sample was plated by using the pour plate method. One ml of each dilution was pipetted into a sterile petri dish and 15-20 ml of medium, cooled down to 45°C, was added. After thorough mixing, the material was allowed to solidify and was incubated for 24 h at

37°C. Purple colonies were counted as *E.coli*, whereas coliforms were giving pink colonies. Results were obtained by multiplying the counted colonies on a petri dish, a readable number between 10-300 colonies, with the dilution factor and were expressed as CFU/ 100 ml.

3.3.1.2. Physico-chemical analysis of water

Temperature, pH, electrical conductivity (EC) and total dissolved solids (TDS) were determined with a pH/ EC/ TDS/ °C/ °F waterproof meter (Hanna Instruments HI-991300) for all water samples.

Water samples, which were obtained from sources, were physico-chemically analyzed at the Laboratory of the Southern Water Resources Development Bureau (SNNPRg) in Hawassa, Ethiopia. The following parameters were assessed with a DR 5000TM UV-Vis Spectrophotometer (Hach, USA): turbidity, Ca²⁺, Mg²⁺, Cu²⁺, Mn²⁺, Cr⁶⁺, F⁻, NO₃⁻ and SO₄²⁻.

3.3.2. Milk

3.3.2.1. Bacterial enumeration in milk

From all milk samples obtained, the following parameters were determined: *Escherichia coli*, total coliform count and total aerobic plate count. A serial dilution with Ringer solution (Oxoid, BR0052) was performed. The samples were diluted up to 10⁻⁴. Quarter samples from each cow were pooled for the initial one ml of the serial dilution by taking 0.25 ml each. If one or more quarters were not milked, the corresponding mixture ratio was applied.

Ergo, *ayib* and yoghurt samples in non-uniform condition were homogenised in a stomacher before diluting.

For *Escherichia coli* and total coliform count BrillianceTM E.coli/ coliform selective agar (Oxoid, CM1046) was used and Plate Count Agar (Standard Methods Agar, HiMedia Laboratories) was applied for enumeration of TAPC.

For both media, the pour plate method was administered. One millilitre of each dilution was pipetted into a sterile petri dish and 15-20 ml of medium, cooled down to 45°C, was added. After thorough mixing, the material was allowed to solidify and was incubated for 24 h at 37°C. One petri dish of every sample with growth between 10-300 colonies was counted and the quantity was multiplied with the corresponding dilution factor. The results were expressed in CFU per millilitre.

3.3.2.2. Bacterial isolation and presumptive identification

For bacteria isolation, samples were plated on blood agar, obtained from sheep blood agar (about 7% sheep blood), by using the quadrant streak method (for details on the agar see Annex 10.1.). After incubation for 24 h at 37°C, individual colonies were characterized and were cultured again on blood agar to achieve pure bacterial colonies. Simultaneously, the milk specimens were cultured on MacConkey agar (Oxoid, CM 0007) and growth (yes-no) and colour were characterized after incubation for 24 h at 37°C.

To differentiate between gram-positive and gram-negative bacteria, colonies were exposed to the Gram staining method after fixation on a slide (for details on the procedure see Annex 10.2). Gram reaction (positive or negative), cellular morphology (rod or coccus) and cellular array (pair, cluster or chain) were assessed under a light microscope (1000x magnification).

For further classification of bacterial genera the following biochemical test were carried out for each isolated colony.

Catalase test

The test is applied for the detection of the enzyme catalase. This enzyme is capable of accelerating the breakdown of hydrogen peroxide to oxygen and water ($\text{H}_2\text{O}_2 + \text{catalase} \rightarrow \text{H}_2\text{O} + \text{O}_2$) and therefore overriding its bactericidal effect. The oxygen production can be easily observed because of immediate bubble formation. On a slide one drop of hydrogen peroxide (3%) is added to a small amount of an isolated colony. Immediate gas production is classified as catalase positive result (American Society for Microbiology, 2010a).

Triple sugar iron (TSI) reaction

Some bacteria ferment specific sugars under formation of acid by- or end products. TSI agar (HiMedia laboratories, M021) contains glucose, sucrose, lactose, pH indicator and ferrous sulphate. Utilization of one of these sugars by a bacterium will lead to a change in colour because of the acid production. Additionally, some bacteria form hydrogen sulphide (H_2S), which reacts with the ferrous sulphate to ferrous sulphide. This is visible in a blackening of the agar (HiMedia Laboratories, 2009a).

Motility test

The motility test is used for differentiation between motile and non-motile bacteria. Semi-solid motility medium (Oxoid, CM0435) in a tube is inoculated with the organism by stabbing a contaminated straight wire once in the media. After incubation at 37°C for 24 hours the tube is examined for bacterial growth. A diffuse growth throughout the media indicates the motility, whereas non-motile organisms are confined to the stab line (Oxoid, 2011).

IMViC test

IMViC comprises four tests and is an abbreviation for indole reaction, methyl red test, Voges-Proskauer test and citrate utilization. The indole reaction is applied for proof of the enzyme tryptophanase, which converts tryptophan to indole. Tryptophan containing media (Oxoid, CM0435) is inoculated with isolated organism and after incubation Kovac's reagent (p-dimethyl aminobenzaldehyde) is added. The produced indole reacts with Kovac's reagent developing a pink colour, which can be observed and read as a positive result (American Society for Microbiology, 2010b).

Methyl red positive bacteria produce strong acid from glucose through the mixed fermentation way. Test organisms are incubated at 37°C for 48 hours on MR-VP media (Titan Biotech Limited, TM 1780) and afterwards five drops of methyl red solution are added. If enough strong acids are produced, a red colour at the surface of the media can be detected and indicates a positive result (American Society for Microbiology, 2010c).

Organisms, which produce acetoin as an endproduct of glucose metabolism, are identified with the Voges-Proskauer reaction. While adding alkali, acetoin is converted to diacetyl, which reacts with arginine in peptone water, producing a pink colour. For the test, bacteria are grown on MR-VP media (Titan Biotech Limited, TM 1780) at 37°C for 24 h. Three ml of 5% α -naphthol, functioning as a catalyser, and one ml of 40% KOH solution is added and vigorously shaken. A positive result is obtained when a pink colour develops (American Society for Microbiology, 2010c).

Citrate test leads to positive results for organism, which are capable of using citrate as a carbon source for metabolism. These bacteria extract nitrogen from ammonium salts as well, which leads to ammonium production and therefore to a change in alkalinity. The test is carried out by incubating the organism on Simmon's citrate agar (HiMedia laboratories, M099), which is containing ammonium salts, at 37°C for 48 hours. Development of deep blue colour within 24-48 hours indicates alkalisation and consequently a positive result (HiMedia laboratories, 2009b).

The results were interpreted according to the procedure described in Quinn et al. (1999) and most probable genera were assigned to every isolated colony.

4. Results

4.1. Water

In total, only 5.88 % of the water samples taken from sources and 4.95% samples originating from households did not exceed any of the threshold₁ values of the examined parameters. Detailed results are given below.

4.1.1. Water microbial

Water samples from sources

E.coli were prevalent in many samples, with means ranging from log₁₀ 2.15 to 6.03 CFU/ 100 ml (Lume) and 2.78 to 5.02 CFU/ 100 ml (Siraro) for the different categories. Total coliform contamination occurred in both districts with averages between 2.40- 6.83 CFU/ 100 ml (Lume) and 3.83- 5.62 CFU/ 100 ml (Siraro). The amount of positive samples for *E.coli* and total coliforms are presented in Table 5. Threshold values for *E.coli* and total coliforms were set to 0 CFU/ 100 ml for both indicators according to the WHO guidelines for drinking water. All samples taken in Siraro revealed bacterial contamination, except the ones obtained from borehole pumps.

Tab. 5 Positive samples for *E.coli* and coliforms of water from sources

District	Source	N	<i>E.coli</i>	Total coliforms
			Number (%)	Number (%)
Lume	River	4	4 (100)	4 (100)
	Industrial discharge	3	1 (33.33)	2 (66.67)
	Hand-dug well	15	5 (33.33)	13 (86.67)
	Borehole pump	2	0 (0)	1 (50)
Siraro	River	2	2 (100)	2 (100)
	Rain	3	3 (100)	3 (100)
	Borehole pump	5	1 (20)	5 (100)

N: Sample size

Water samples from storage container in households

Water samples from storage containers in households were assigned to categories according to the source, which they initially originate from. Averages in CFU/ 100 ml are presented in Figure 2, comparing Lume and Siraro.

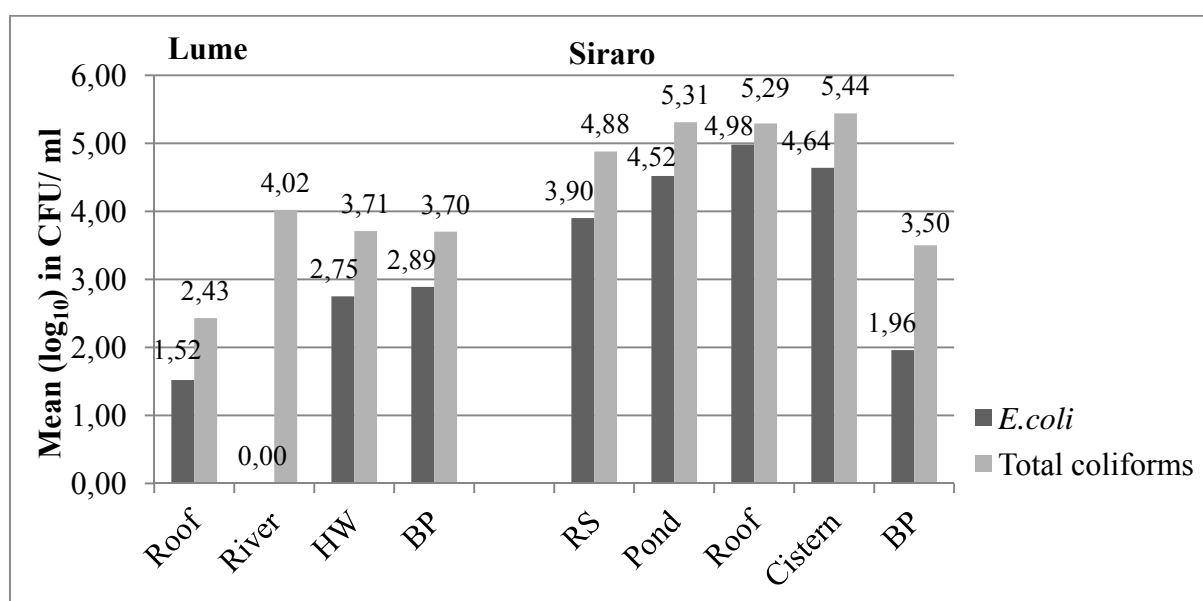


Fig. 2 Mean values of the bacterial counts of water from households

HW: hand-dug well; BP: borehole pump; RS: roadside

The drinking water in households was contaminated with *E.coli* and other coliforms regardless of the initial source. In Siraro mean values were generally higher than in Lume.

4.1.2. Water physico-chemical

Water samples from sources

Table 6 summarizes the quantity of contaminated samples (%) for different water sources in Lume and Siraro. The recommended standards by the World Health Organization (WHO) served as threshold values (WHO, 2011). Borehole pumps in Lume were not above the standards for any of the assessed parameters, whereas water from industrial discharges contained several chemical contaminants in excessive amounts. Concentrations of magnesium, copper, or nitrate were of no concern according to the standards by the WHO in samples from both Lume and Siraro. Maximum fluoride concentration was 6.9 mg/ l for borehole pumps in Siraro.

Tab. 6 Physico-chemical quality of water from sources (in % exceeding WHO thresholds)

WHO		Lume				Siraro		
threshold		River	ID	HW	BP	River	RP	BP
N		4	3	15	2	2	3	5
pH		no WHO guideline available (recommended 6.5-8.5)						
Temp.		no WHO guideline available						
EC		no WHO guideline available						
TDS	1000 mg/l	0	100	0	0	0	0	0
Turbidity	5 NTU	n.m.	n.m.	46.7	0	n.m.	n.m.	20.0
Ca ²⁺	100 mg/l	0	100	13.3	0	0	0	0
Mg ²⁺	100 mg/l	0	0	0	0	0	0	0
Cu ²⁺	2 mg/l	0	0	0	0	0	0	0
Mn ²⁺	0.4 mg/l	0	66.7	26.7	0	n.a.	33.3	60.0
Cr ⁶⁺	0.05 mg/l	0	66.7	6.7	0	50.0	33.3	20.0
F ⁻	1.5 mg/l	0	n.a.	20.0	0	n.a.	0	100
NO ₃ ⁻	50 mg/l	0	0	0	0	0	0	0
SO ₄ ²⁻	250 mg/l	0	100	0	0	0	0	0

ID: Industrial discharge, HW: hand-dug well, BP: borehole pump, RP: rainpond, n.m.: not measurable, n.a.: not analysed

PH of industrial wastewaters was generally alkaline (mean 9.88) with a maximum of 11.55. All rivers in Lume (mean 9.09) were more basophile than the recommended pH value of 8.5. Two out of three samples obtained from collected water of roofs in Siraro were below the minimum recommended value of 6.5. All other sources ranged between pH 6.5 and 8.5.

Temperature differed for the categories with mean values between 16.9°C (roof Siraro) - 26.5°C (industrial discharge Lume). Maximum was measured in a borehole pump in Siraro (35.7°C).

Average electrical conductivity was low for rivers in Siraro (73.5 μS) and Lume (374.3 μS) and water from roofs (90.7 μS), whereas borehole pumps in Siraro (618.8 μS) and Lume (598.0 μS) as well as hand dug wells in Lume (730.5 μS) showed higher values. Industrial discharges went beyond the technical measurable maximum of 4000 μS .

The determination of total dissolved solids revealed no elevated concentration for any of the investigated sources, except industrial wastewater, which exceeded technical measurable maximum of 2000 mg/ l. In general, rivers and rain water gave lower results (mean 37-191 mg/ l) in comparison to borehole pumps and hand dug wells (mean 298-373 mg/ l).

Turbidity was not recorded for industrial discharges and rivers owing to limited technical advices. Hand dug wells were polluted in 46.7 % of the cases averaging 36 NTU.

Water samples from storage containers in households

Table 7 summarize the assessed physico-chemical parameters for water attained from households in Lume and Siraro. Samples are assigned to categories depending on the original source. For temperature and electrical conductivity (EC) there is no existing WHO guideline. None of the samples was exceeding the threshold value of 1000 mg/ l for TDS.

PH measurement generally gave results between 6.5 and 8.5; however 17.9 % of the borehole pumps in Lume, 11.8 % of the ponds, 4.2 % of the rain water sources and 11.1 % of the water from roadsides in Siraro showed a pH of 6.5. One out of 15 cisterns in Siraro went beyond 8.5.

Temperature ranged from 16.4°C up to 29.7°C. Electrical conductivity seemed to be higher in borehole pumps and hand-dug wells compared to surface water (river, pond, roof and cistern).

Tab. 7 Physico-chemical quality of water from households

	N	pH	T (°C)	EC (μS/cm)	TDS (ppm)
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
River Lume	1	8.66 (0)	23.30 (0)	95 (0)	48 (0)
Roof Lume	3	7.02 (0.12)	23.57 (1.33)	22 (20)	11 (9)
Hand-dug well Lume	12	7.43 (0.23)	19.68 (1.65)	611 (65)	311(33)
Borehole pump Lume	28	6.88 (0.51)	21.95 (3.29)	312 (191)	158 (97)
Roadside Siraro	9	7.11 (0.52)	18.37 (1.10)	66 (69.79)	33 (35.54)
Pond Siraro	17	6.88 (0.42)	18.67 (1.75)	48 (49.35)	24 (25.14)
Roof Siraro	24	7.27 (0.53)	19.02 (1.64)	35 (66.75)	17 (33.91)
Cistern Siraro	15	7.14 (0.50)	18.65 (1.14)	107 (98.59)	54 (50.13)
Borehole pump Siraro	12	7.97 (0.30)	19.32 (1.51)	277 (320.81)	141 (163.32)

T: Temperature, EC: Electrical conductivity, TDS: Total dissolved solids, SD: Standard deviation

4.2. Milk

4.2.1. Milk quantitative

At all analyzed points of the milk production chain, contamination with *E.coli* occurred. The numbers of milk samples, which were tested positive for *E.coli* or total coliforms with the threshold value of 0 CFU/ ml and 10 CFU/ ml respectively, are shown in figure.

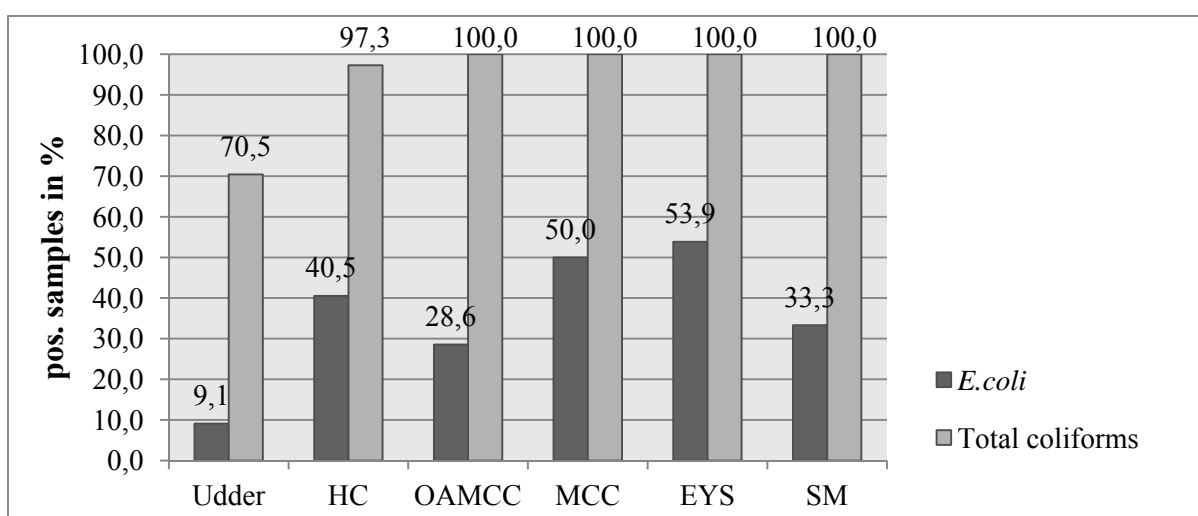


Fig. 3 Percentages of samples positive for *E.coli* and coliforms of milk products

HC: household container; OAMCC: on arrival milk collection centre; MCC: milk collection centre; EYS: *ergo*, yoghurt, *ayib*; SK: skim milk

E.coli was prevalent in the range up to $10^{-5.43}$ CFU/ ml in ready-to-eat milk products (raw milk from storage containers of households, *ergo*, yoghurt, *ayib*, skim milk and storage containers at milk collection centre). Mean values of the determined bacterial counts are presented in Table 8. Udder milk already contained high amounts of bacterial counts. Both, total coliform count and SPC increased on the way to the milk collection centre. The processed products (*ergo*, yoghurt, *ayib* and skim milk) contained *E.coli* and also high numbers of total coliforms.

Tab. 8 Bacterial count of milk samples from different points of the production chain

	Point of sampling					
	Udder	HC	OAMCC	MCC	EYA	SM
	(CFU/ ml)	(CFU/ ml)	(CFU/ ml)	(CFU/ ml)	(CFU/ ml)	(CFU/ ml)
	Mean (log ₁₀)					
Sampling size	44	37	7	2	13	6
<i>E.coli</i>	2.36	2.89	1.20	2.78	4.42	3.02
Total coliforms	4.85	5.42	5.78	6.23	5.75	5.15
SPC	4.81	5.26	5.50	6.06	5.29	4.76

HC: storage container household; OAMCC: on arrival milk collection centre; MCC: storage container milk collection centre; EYA: *ergo*, yoghurt, *ayib*; SK: skim milk

In the majority of cases, SPC was less than 1000 CFU/ ml for milk directly taken from the udder (59.09%). All of the samples from household containers were assigned to the SPC grade ‘very good’ (78% < 200.000 CFU/ ml) or ‘good’ (22% 200.000-1.000.000 CFU/ ml). On arrival to milk collection centre, three out of seven samples belonged to SPC grade ‘fair’ (1.000.000- 5.000.000 CFU/ ml).

4.2.2. Milk qualitative

Out of 169 quarter milk samples obtained from 44 cows 255 colonies were isolated and identified. Some quarters (25 out of 169) did not display any bacterial growth. Figure 4 illustrates the determined genera and the corresponding percentages.

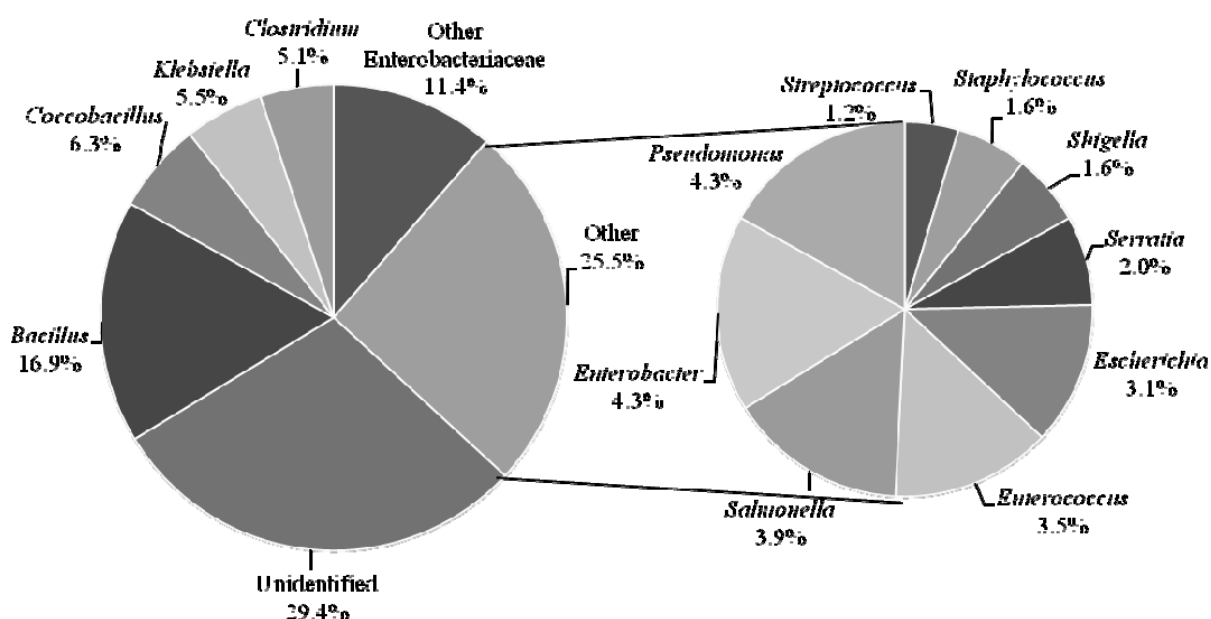


Fig. 4 Identified genera in milk from quarters

With the applied biochemical tests, 75 (29.41 %) colonies could not be specified. For 29 colonies (11.37 %) characterisation could be done up to the family of Enterobacteriaceae.

For pooled raw milk samples, including those taken from storage containers in households, transport containers and storage containers at the milk collection centre, 49 samples were taken, resulting in 104 colonies. In Figure 5 the main genera are summarised. Additionally, one sample (0.001%) of *Citrobacter* spp., *Coccobacillus* spp, *Salmonella* spp. and *Serratia* spp. respectively was characterised.

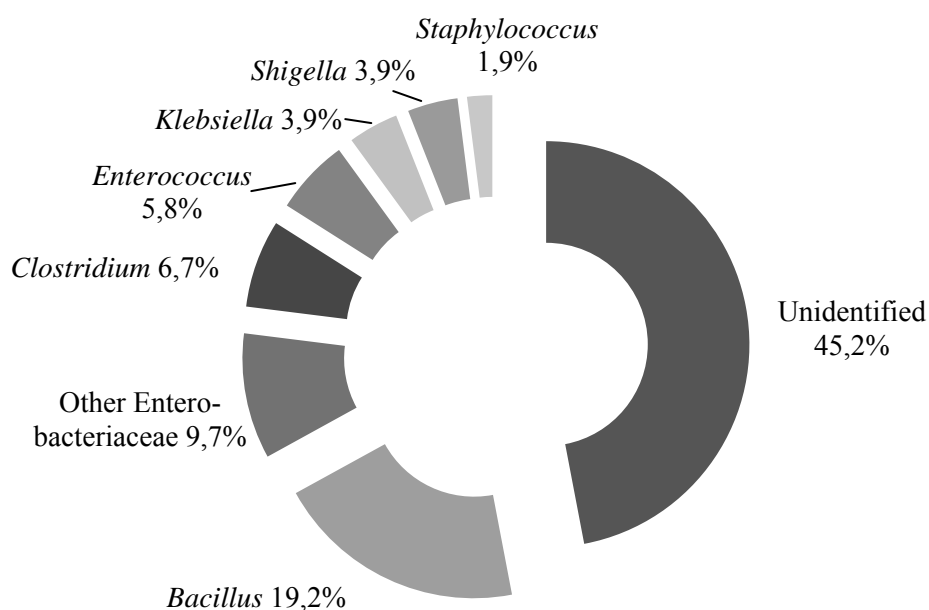


Fig. 5 Main bacterial genera identified from pooled raw milk samples

Ergo, yoghurt, *ayib* and skim milk were combined to the category of “other milk products”. In total 41 colonies were specified out of 19 samples. Figure 6 gives the identified genera and corresponding percentage.

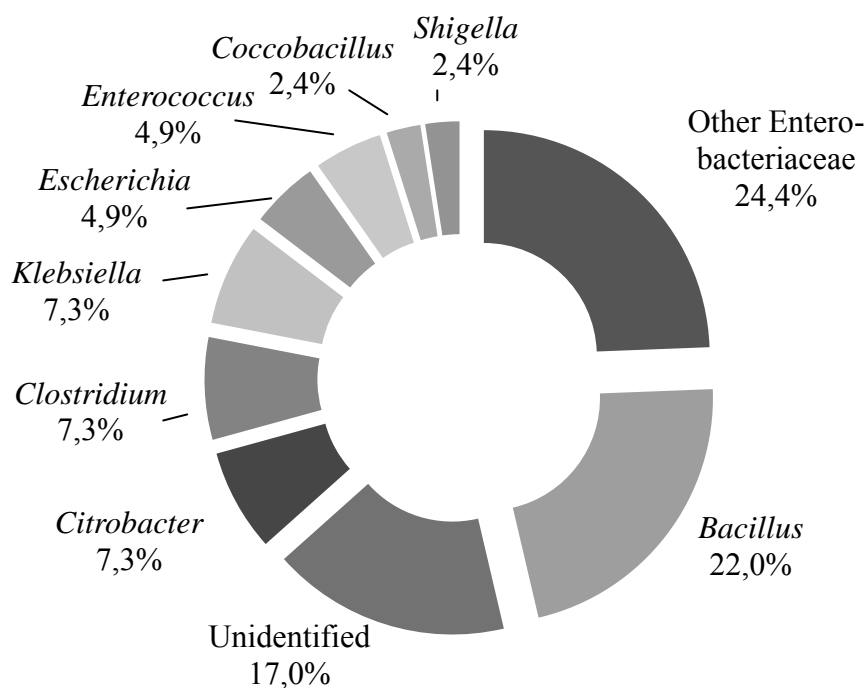


Fig. 6 Bacterial genera identified from "other milk products"

Every sample showed growth of at least one bacterium. Seven colonies could not be determined at all and ten were only characterised up to the family of Enterobacteraceae.

5. Discussion

Drinking water aspects

With regard to drinking water, the purpose of this study concerning was to identify deficiencies in quality, which are potentially capable of adversely effecting health. In general, neither physico-chemical and bacterial quality was acceptable on source, nor on household level, except for borehole pumps in Lume.

The pH value of industrial wastewater is often basophile, which was confirmed by the results of the present study regarding industrial discharges in Lume. All sampling places for rivers in this district were located close to tanneries, abattoirs or small towns. As it is the local custom sewages are released unfiltered into rivers, which is likely to be the main reason for alkaline pH values in rivers. Causes for acidity in water from natural sources are usually due to calcareous soils or acid rain as an effect of air pollution (Erah et al., 2002). The latter may accounts for low pH values in rain water sources in Siraro and consequently in water samples from households, which are fetched from roadsides or collected directly from the roof. However, up to date there are no studies suggesting or confirming air contamination in respective districts. Both aspects might contribute to low pH in borehole pumps or ponds. In general, it is assumed that pH values from boreholes are lower than those from wells and rivers (Nduka et al., 2008), which is partly approved by the present study.

High temperatures in borehole pumps in Siraro might be explained by the heat loss of the applied technical devices. In water storage containers temperature is mainly influenced by the degree and period of exposure to the sun, leading to fluctuating results for households.

Electrical conductivity indicates the total concentration of salts and depends basically on soil type and import of wastewater (Erah et al., 2002), giving reasons for low values in sources from surface water, but higher results for groundwater and excessive values for industrial discharges.

All TDS samples were below the guideline, except for water from industrial discharge, indicating a high level of pollution.

All other chemical parameters were only analyzed for water directly from sources, due to the assumption, that contamination between ladling and consumption is negligible and may only occur as a result of pooling water from different sources.

Previous studies found, that turbidity is lower for borehole pumps than for hand-dug wells and rivers (Nduka et al., 2008), which complies with the outcome of this survey. Run-offs from particles of soil after heavy rains may explain the not measurable values with the applied advices in rivers of Lume, (Zamxaka et al., 2004) because this district shows elevated rainfall during the rainy seasons from July to September representing the sampling time. Other causes might be poor farming practices in the districts, such as overgrazing, leading to topsoil erosion, which is washed down in waters (Nevondo & Cloete, 1999). Half of the hand-dug wells revealed high turbidity, likely due to building materials, which are susceptible for corrosion and degradation over time.

No elevated amounts of magnesium, copper and nitrate were found in the present study. Calcium, manganese, chromium and fluoride concentrations are basically affected by (pedogenic) rocks and consequently depend on the region. Erah et al. (2002) found higher values of chromium in wells and borehole pumps, owing to the groundwater composition (Erah et al., 2002). Sulphate indicates contamination from industrial sewage and excessive amounts were especially found in those from tanneries and paper mills (Nduka et al., 2008), which coincide to the results of industrial discharges in Lume, where two tanneries effluents were sampled. Notification needs to be given to local authorities when concentrations higher than 500 mg/ l are recorded (WHO 2011, p. 419), which occurred in this study in one case.

Guidelines for drinking water of the WHO (WHO 2011) and several studies illustrate, that pH, temperature and turbidity of water from sources affect microbial growth. Basophile pH in industrial discharges might have prevented further growth of *E.coli* and other coliforms. All rivers were contaminated with bacteria, which may be influenced by the corresponding high turbidity results, supplying many nutrients.

One study revealed, that groundwater is most often better in microbial quality than surfaces water (Zamxaka et al., 2004), which is partly confirmed by this study; however, some contamination with *E.coli* and other coliforms was found in boreholes and hand-dug wells. *E.coli* is an indicator for human or animal faeces and total coliforms represent poor sanitation and further poor disinfectant treatment. It may occur, that small amounts of excreta from latrines or other contaminants leach into groundwater and are finally detected in wells and boreholes (Erah et al., 2002; Nevondo & Cloete, 1999).

Defects related to plumbing in water supply systems induce microbial contamination and growth as well (Nevondo & Cloete, 1999).

High bacteria counts in rivers might be caused by human and animal activities around the sources (Zamxaka et al., 2004). It was observed, that people used the water from rivers not only for drinking but also for bathing, washing and recreation. Owing to wild and domestic animals drinking from rivers and thereby standing in the water, because there is no protection of the water source, bacterial contamination is higher. Zamxaka et al. (2004) claimed that flowing waters result in lower bacterial counts than stagnant waters, such as ponds and cisterns; however, no differences between rivers and ponds could be found in the present study.

Rain water is commonly assumed to be naturally pure and free of microbes. Contamination with *E.coli* and other coliforms in collected rain water most likely occurs therefore through input of “organic matter such as leaves, insects and bird droppings, plus small amounts of dirt and dust” (Nevondo & Cloete, 1999).

On a household level, microbial water quality is basically influenced by the initial prevalence of bacteria and the physical state of the water sources, the storage conditions, fetching and handling practices supporting microbial growth as well as the applied treatment methods to improve quality. The original bacterial load from sources was shown to be high in Siraro and impaired in Lume as well. Physical water conditions, such as turbidity were also poor. Both aspects may represent weak starting points for the bacterial water quality, even worse for Siraro. Moreover, only few households apply treatment methods like boiling, filtration or disinfection with chemicals in the investigated districts, which can account for unpalatable conditions. All households used plastic jerry cans for keeping water, which were exposed more or less to the sun. In combination with high turbidity, these structures are utterly suitable for microbial growth.

Alltogether, bacterial water quality of especially drinking water at the place of consumption depends on local dominating prerequisites, such as supply with improved water sources, sanitation and hygiene conditions, the environmental awareness of people and finally human and animal activities around the water sources (Zamxaka et al., 2004). Local differences between Lume and Siraro could be found in present study, owing to the fact that people in Siraro have less access to developed water sources than the population in Lume.

Milk aspects

Regarding the milk aspect of the present study it was the objective to determine the quantitative and qualitative bacterial prevalence at the different points along the value chain to get an overall idea of critical factors in milk production and the risk of health at the point of consumption. Milk directly obtained from the udder already showed high amounts of total coliforms and around 40% of the cows displayed elevated total plate counts. This is most likely due to mastitis. In total, in two of 44 cows lesions of the teats could be observed and one farmer reported an infection of the mammary gland a few weeks ago. Mekibib et al. (2010) determined 44.9% of mastitis cases on a quarter level in central Ethiopia, whereby the majority was found to be subclinical (Mekibib et al., 2010). Several other studies confirmed that mastitis occurs in around 30-40% of the cows in Ethiopia and suggested that most of them do not show clinical symptoms (Biffa et al., 2005). It can be therefore assumed that despite the fact that only three of 44 samples were suspicious to infections due to visible signs, a much higher percentage of cows was infected with subclinical mastitis. In addition, 86% of the examined cows were hybrids of local breeds with Holstein-Friesians, which are more susceptible to inflammations of the udder than indigenous breeds. The wet season in Ethiopia is also positively correlated to a higher mastitis incidence rate (Biffa et al., 2005). *E.coli* was identified to be present in infected cows (Mekibib et al., 2010), leading to the suggestion that the prevalence in nine percent of the samples is most probable connected to mastitis as well.

In general, several studies concluded that bacterial counts increase from farmers reaching consumers when no treatment for reduction is applied. This is owing to the fact that possible sources of microorganisms are added up with the addition of people handling the product, of containers and of different environmental effects. Furthermore time is a crucial factor (Grimaud et al., 2007; Bonfoh et al., 2003).

The number of samples that were stored in containers in households were tested positive for *E.coli* and total coliforms increased, compared to the ones taken directly from the udder. The result of 97.3% for total coliforms was slightly higher than in comparable studies, for example one from Malaysia, which found 89.9% positive cultured raw milk samples from dairy farmers (Chye et al., 2003). However, the corresponding results for *E.coli* prevalence indicated less contamination. In Uganda high SPC, total coliform and *E.coli* counts were already determined at the farm as well (Grimaud et al., 2007). Several sources of contamination were identified comprising the initial bacterial load from cows infected with

mastitis and poor production conditions. Most farmers kept their cows inside a cow byre, its ground covered with dung or soil occasionally, which are known to contain bacteria capable of migrating over the udder surface into milk. Furthermore milking management, which was reported by farmers, appeared to be weak. The udder was cleaned with dry hands or water, the fist squirts, which contain the highest amount of bacteria in milk, were not discarded and no teat dipping was applied. However, no correlation between the applied material for cleaning the udder and the microbial load was detected in a study in Zimbabwe (Gran et al., 2001). It was found that not tying the cow's tail while milking may be a contamination factor as well (Grimaud et al., 2007).

The personal hygiene of people handling milk is essential. It was observed that stuff frequently cleaned their hands only with cold water before milking. Gran et al. (2001) recognised that the results for bacterial counts are higher when hands that had come in contact with the ground or an animal while milking and were put into the milking vessel without previous washing. However, the every-day behaviour of the individual persons in the present study is not undoubtedly known.

The type of storage containers, cleaning practices of the milking equipment and storages conditions, such as temperature and the time until consumption or selling, are known to be crucial. In both districts milk was not cooled in any household. Chye et al. (2003) stated that "utensil used for milking should be rinsed, cleaned using detergents and disinfected immediately after use" to lower prevalence of bacteria in milk on the farm level. In addition, in one study it was suggested that the microbial quality of water used for cleaning and applied detergents and disinfectants affect the likelihood of bacterial contamination (Grimaud et al., 2007). As the bacterial quality of water in households was assessed to be very poor in the present study, this may be a further contamination source. On the other hand there was another survey, which could not find any correlation between bacterial load of water and milk, though (Gran et al., 2001). The same paper concluded that the quality of milk on farm level was relatively good despite several deficiencies in milk production management. This is in compliance with the findings in Lume and Siraro, regarding the percentages of samples assigned to SPC grade 'very good' (78%) and 'good' (22%) and the observed poor conditions.

On arrival to the milk collection centre the quality parameters were further deteriorated. All samples were cultured positive for total coliforms and three out of seven samples were assigned to SPC grade 'fair'. Almost one third of the farmers supplied the centre with milk contaminated with *E.coli*. This is in line with a study determining transport and milk

collection as the most crucial effects for sources of and supporting factors for bacterial growth (Afif et al., 2008). The distance from farm to centre and the time for transportation was shown to correlate with bacterial concentrations (Gran et al., 2001). Farmers delivered daily in the afternoon to the collection centre, carrying their milk in plastic or metal cans. Thus the increase in bacteria numbers are mainly due to the unchilled conditions while transportation, an additional vessel used for storage and more people, which are involved in handling milk, as possible sources of contamination (Grimaud et al., 2007).

Further critical points at the level of milk collection centres were noticed to be the absence of quality assessment methods on arrival and the number of delivering farmers to centre. In Lume and Siraro many small-scale farmers exist. Consequently the milk yields of numerous farms are pooled at the collection centre. As it is known, that only small amounts of bacterial contaminants lead to rapid multiplication (Afif et al., 2008) this is a crucial factor. In addition, raw milk was not stored cool between arrival and subsequent selling or processing.

Ergo, *ayib* and yoghurt samples could be expected to have lower mean bacterial counts and less positive samples due to thermal treatment and low pH while processing. Ashenafi (1990) analysed 55% positive culturing samples of *ayib* in Ethiopia. In comparison, the present study revealed that all inspected milk products, including *ergo*, *ayib* and yoghurt, were tested positive for coliforms and showed consistently high *E.coli* counts (mean $10^{4.42}$ cfu/ ml). The most possible sources of recontamination after processing are people handling the products, parts of plants, which are used for packing and herbs, which are added for flavour (Aschenafi, 2006). For the investigated products packing and intermixed herbs were not applied, leading to the assumption that people or utilized equipment functioned as a carrier for bacteria.

The most abundant genera, which were isolated from all milk products were *Bacillus* and *Clostridium*. Several other studies determined high amounts of them as well (Chye et al., 2003). They belong to the group of psychotrophic bacteria, growing at low temperatures and are mainly responsible for spoilage of milk (Chye et al., 2003). It is stated that psychotrophs can account for more than 75% of the microbial flora in milk samples, which were obtained under unhygienic conditions. Other genera belonging to the group of psychotrophs and which were identified in the present study are *Pseudomonas*, *Enterobacter*, *Serratia*, *Staphylococcus* and *Streptococcus* in the range of their percentage beginning with the most frequent one. Commonly *Pseudomonas* spp. are known to be the most prevalent species within the psychotrophs (Hantsis- Zacharov & Halpern, 2007); however they were of minor concern compared with *Bacillus* spp. and *Clostridium* spp. in the surveyed samples. *Bacillus* spp. in

high concentrations can be pathogens (Huppertz & Kelly 2009, p. 42). The establishment of sanitary conditions and practices are essential to reduce contamination with psychotropic organism.

The third common genus occurring in milk was *Klebsiella* spp., which was found to be connected to mastitis in cows. Moreover, *Streptococcus* spp., *Escherichia* spp., *Enterobacter* spp., *Bacillus* spp. and *Staphylococcus* spp. were isolated from samples. These genera were identified in milk from infected cows as well. But in most of the cases *Staphylococcus aureus* was indicated as the pathogen (Mekibib et al., 2010). *Staphylococcus* spp. was detected in udder milk in only 1.57% of the colonies, which is much lower than in other studies, where percentages up to 60% and more were specified (Chye et al., 2003).

In addition, genera including pathogens, which are known to cause severe diseases, were *Staphylococcus*, *Streptococcus*, *Escherichia* and *Salmonella* in milk samples from the udder and pooled raw milk. However, this does not need to implicate immediate disease outbreaks among consumer, because these genera may comprise both pathogenic and non-pathogenic species and strains (Soomro et al., 2002).

6. Conclusion

As it was revealed that only the minority of water samples from both sources and households was in a state of good quality and safe to consume there is a demand for action. The villages in Lume and Siraro are representative for many other districts in Ethiopia and developing countries in general, where water is either scarce or polluted by industry or public. Water consumption poses a hazard to health of humans, which is not only influencing the welfare of the consumer but also socio-economic aspects. The improvement of the supply of people in rural parts of the country with good quality water is essential for sustained economic growth and consequently for further development in a long-term. The provision of improved water sources, which is the responsibility of the government and the education of consumers concerning sanitation and water handling, can be approaches for advancement in water quality, for example.

As aforementioned, the applied water for cleaning equipment in milk production may influence the quality of the end-product. In the present study, several sources of bacterial contamination were identified. Further analyses are necessary to investigate the correlation between water and milk quality.

Despite the impaired microbial quality of the consumed milk products, up to date there was no severe disease outbreak related to milk consumption in the surveyed districts. To ensure that milk is safe to consume it is crucial to improve milk handling on the specific sites along the production chain. The education of farmers in hygienic milking management and milk storage, the implementation of quality-paid sale and the training of consumers regarding treatment methods for milk are examples for strategies in quality advancement of milk.

7. Summary

The provision with water of good quality is essential, due to the fact that every person is depending on it and the knowledge of several water-borne diseases. The transmission of pathogens is possible by the consumption of raw milk and milk products as well. However, water and milk quality is not necessarily monitored, especially in rural parts of developing countries like Ethiopia. The present study was performed to determine the quality of water and milk at the point of production and in comparison at the place of consumption.

Water samples were taken from the commonly used sources in Lume and Siraro, which are rivers, rainwater ponds, cisterns, roof water, hand-dug wells and public borehole pumps. The physico-chemical parameters (pH, temperature, electrical conductivity, total dissolved solids, turbidity, Ca^{2+} , Mg^{2+} , Cu^{2+} , Mn^{2+} , Cr^{6+} , F^- , NO_3^- and SO_4^{2-}) as well as the bacterial content of *Escherichia coli* and total coliforms were determined for these specimens. The enumeration of the aforementioned bacteria was also done for water samples obtained from containers of water in private households.

Milk was sampled directly from the udder and along the milk production chain from storage containers of raw milk in households, from transport containers on arrival to the milk collection centre and from storage containers at the milk collection centre. Moreover, samples of *ergo*, a traditional fermented milk product similar to sour milk, *ayib*, a locally produced soft cheese, yoghurt and skim milk were obtained. The counting of *Escherichia coli* and total coliforms and the total aerobic plate count was performed as well as the isolation and identification of the bacterial genera. For the determination of the genera, gram staining and several biochemical tests (catalase test, TSI reaction, motility and IMViC test) were applied.

Only 5.88% and 4.95% of the water samples from sources and households, respectively, did not exceed any of the recommended threshold values for drinking water by the World Health Organization and were consequently regarded to be safe for consumption. The borehole

pumps in Lume showed the lowest contamination and industrial discharges were highly polluted. These sewages are released into rivers without any filtration.

The analysis of all milk samples revealed high values of *Escherichia coli*, total coliforms and total aerobic plate counts. *E.coli* bacteria were already found in the milk obtained from the udder. The average of colony forming units per ml (CFU/ ml) for all determined bacteria increased slightly along the production chain. The processed products (*ergo*, *ayib* and skim milk) showed consistently high amounts of *Escherichia coli* and total coliforms.

Bacterial genera, which are mainly responsible for spoilage in milk, were identified most often. Some of the specimens were contaminated with pathogenic species, like *Staphylococcus* spp., *Streptococcus* spp., *Escherichia* spp., and *Salmonella* spp.

It can be summarized, that in general neither the water quality nor the milk quality was acceptable for direct consumption. As a consequence drinking of water or eating of milk in Lume and Siraro poses a risk for health. Consumers as well as the government can and should take measures to improve the quality.

8. Zusammenfassung

Die Sicherstellung einer Versorgung mit Wasser von guter Qualität ist enorm wichtig, da zum Einen jeder Mensch davon betroffen ist und zum Anderen bekannt ist, dass durch Pathogene oder Chemikalien in Wasser erhebliche gesundheitliche Folgen möglich sind. Auch durch den Verzehr von Rohmilch und Milchprodukten kann es zur Übertragung von Krankheitserregern kommen. Vor allem in ländlichen Gebieten von sich entwickelnden Ländern wie Äthiopien, ist die Überwachung der Wasser- und Milchqualität meist nicht gegeben. Diese Studie wurde durchgeführt um die Qualität von Wasser und Milch am Ort ihrer Gewinnung und vergleichend bei Aufnahme bzw. Verzehr durch den Verbraucher zu beurteilen.

Wasserproben von den in Lume und Siraro üblichen Quellen, das bedeutet von Flüssen, Regenteichen, Zisternen, Regenauffangbehältern, handgegrabene Brunnen und öffentliche Tiefbrunnen, wurden sowohl auf physikalische und chemische Eigenschaften (pH, Temperatur, elektrische Leitfähigkeit, Gesamtgehalt gelöster Stoffe, Trübung, Ca^{2+} , Mg^{2+} , Cu^{2+} , Mn^{2+} , Cr^{6+} , F^- , NO_3^- , SO_4^{2-}) als auch auf Bakteriengehalt (*E.coli* und gesamt koliforme Bakterien) untersucht. Aus Wasseraufbewahrungsbehältern in Haushalten wurden ebenfalls Proben genommen und anschließend auf ihren Bakteriengehalt analysiert.

Für Milch, die direkt aus dem Euter stammt, und verschiedene Produkte der Milchverarbeitung, nämlich Rohmilch, *ergo* (traditionell hergestellte Sauermilch), *ayib* (lokaler Frischkäse) und Magermilch, wurde Gehalt und Zusammensetzung der Bakterien bestimmt. Hierfür wurden die Kolonien-bildende Einheiten (CFU) für *E.coli*, koliforme Bakterien und die Gesamtheit aerober Bakterien, nach Inkubation auf Selektivmedium, gezählt. Die Bestimmung der Bakteriengattungen geschah auf Grundlage von Gramfärbung und verschiedener biochemischer Tests (Katalase Test, TSI Reaktion, Motilität, IMViC Test). Nur 5.88% bzw. 4.95% der Wasserproben von Quellen bzw. Haushalten lagen für alle gemessene Parameter unter den, von der *World Health Organization* geforderten Höchstwerten für Trinkwasser. Dabei wies Wasser aus Tiefbrunnen in Lume die geringste Kontamination auf. Vor allem Abwässer der Industrie, die ungefiltert in Flüsse eingeleitet werden, waren stark verschmutzt.

Die Analyse der Milchproben an den einzelnen Stellen der Produktionskette ergab durchgängig hohe Werte für *E.coli*, koliforme Bakterien und die Gesamtheit aerober Bakterien. Schon Proben aus dem Euter waren mit *E.coli* kontaminiert. Die durchschnittliche Anzahl an CFU/ ml stieg für Rohmilch aus den einzelnen Aufbewahrungsbehältern (Haushalt, Transport und Milchsammelstelle) zum Verbraucher hin an. Die anderen Milchprodukte (*ergo*, *ayib* und Magermilch) zeigten keine Verringerung der bakteriellen Population durch die Verarbeitung.

Am häufigsten wurden Bakteriengattungen, die hauptsächlich für den Verderb der Milchprodukte verantwortlich sind, identifiziert. Einige Proben waren mit Gattungen, die pathogene Arten beinhalten, wie *Staphylococcus*, *Streptococcus*, *Escherichia* und *Salmonella* kontaminiert.

Zusammenfassend ist zu sagen, dass im Allgemeinen weder die Wasserqualität noch die Milchqualität in den Gebieten Lume und Siraro zufriedenstellend ist. Folglich geht mit dem Konsum von Wasser und Milch ein erhebliches Gesundheitsrisiko einher. Maßnahmen zur Verbesserung können und sollten sowohl vom Verbraucher als auch von öffentlicher Seite ergriffen werden.

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Table

Tab. 3: Kurwijila, R.L., K.K. Hansen, I.E. Macha, K. Abdallah and J.H.S. Kadigi (1992): The bacteriological quality of milk from hand machine milked dairy herds in Morogoro, Tanzania. *African Livestock Research* (1992) 2, pp. 59-67.

10. Annexes

10.1. Applied media for bacterial enumeration and isolation

(Sheep) Blood agar base (Oxoid, CM0854)

Composition:	Tryptone	14.0 g/l
	Peptone neutralised	4.5 g/l
	Yeast extract	4.5 g/l
	Sodium chloride	5.0 g/l
	Agar	12.0 g/l

Preparation: In one litre of distilled water 40g of blood agar base are suspended. The solution is boiled to dissolve the powder completely. The medium is sterilised by autoclaving at 121°C for 15 minutes. After cooling down to 45-50°C, 7% sterile blood is added and poured in sterile petri dishes.

MacConkey agar (Oxoid, CM0007)

Composition:	Peptone	20.0 g/l
	Lactose	10.0 g/l
	Bile salts	5.0 g/l
	Neutral red	0.075 g/l
	Agar	12.0 g/l
	pH	7.4 ± 0.2

Preparation: In one litre of distilled water 52 g of MacConkey agar are suspended. The solution is boiled to dissolve the powder completely. The medium is sterilised by autoclaving at 121°C for 15 minutes. Before inoculation the surface of the gel is dried.

Plate count agar (Standard method agar)(HiMedia laboratories, M091)

Composition: Casein enzymic hydrolysate 5.0 Gms/ l

Yeast extract 2.5 Gms/ l

Dextrose 1.0 Gms/ l

Agar 15.0 Gms/l

pH 7.0 ± 0.2

Preparation: In one liter of distilled water 23.5 g of Plate count agar are suspended. The solution is heated until boiling and powder is completely dissolved. The medium is sterilized by autoclaving at 121°C for 15 minutes.

Brilliance E.coli/ coliform selective agar (Oxoid, CM1046)

Composition : Peptone 8.0 gm/ l

Di-sodium hydrogen phosphate 2.2 gm/ l

Sodium chloride 5.0 gm/ l

Potassium di-hydrogen phosphate 1.8 gm/ l

Sodium lauryl sulphate 0.1 gm/ l

Chromogenic mix 0.35 gm/ l

Agar 10.6 gm/ ml

pH 6.7 ± 0.2

Preparation: In one liter of distilled water 28.1 g of agar are suspended. The medium is heated until boiling. For the pour plate technique the media is kept at 45°C.

10.2. Gram-staining procedure in detail

Reagents:

Crystal violet:	Crystal violet	2 g
	Ethanol (95% vol/vol)	20 ml
	Ammonium oxalate	0.8 g
	Distilled water	80.0 ml

First of all the crystal violet is dissolved in the ethanol. Simultaneously the ammonium oxalate is dissolved in distilled water. Both solutions are added together and stirred in a hot bath of water.

Gram's iodine:	Iodine crystals	1 g
	Potassium iodine	2 g
	Distilled water	200 ml

Iodine crystals and potassium iodine are powdered in a mortar and distilled water is added.

Carbol fuchsin: 10 ml of concentrated carbol fuchsin is diluted with 90 ml of distilled water.

Safranin:	Safranin	2.5 g
	Ethanol (95% vol/ vol)	100 ml

The Safranin is dissolved in Ethanol and diluted with distilled water in the ratio 1:4.

Procedure:

Put the slide on a staining rack over a sink.

Add crystal violet on the slide for 30 seconds.

Rinse with tap water.

Pour iodine solution on the slide for 30 seconds.

Rinse with tap water.

Wash the slide with Ethanol (95% vol/ vol) for 10 seconds.

Rinse with tap water.

Pour safranin on the slide for 30 seconds.

Rinse with tap water.

Air-dry and survey under a microscope (magnification 1000×) for gram reaction (+ or -), morphology (coccus or rod) and cellular order (pair, cluster, chain). Gram positive bacteria appear purple whereas gram-negative bacteria are visible in pink.

Declaration*

I,

Name, First name Spengler Marisa

Born on 11.06.1989

Matriculation number 443036

hereby declare on my honor that the attached declaration

- ☐ Homework/ Presentation
- ☐ Bachelor Thesis
- ☐ Master Thesis
- ☐ Diplom Thesis,

has been independently prepared, solely with the support of the listed literature references, and that no information has been presented that has not been officially acknowledged.

Supervisor

Lecturer Prof. Dr. Anne Valle Zárate

Thesis topic Assessment of water and milk quality in rural mixed crop-livestock farming systems: a case study of Lume and Siraro districts, Ethiopia

Semester Wintersemester 2011/ 2012

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